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New Therapeutic Strategy for Gastric Carcinoma: A Two-Step Evaluation of Malignant Potential from Its Molecular Biologic and Pathologic Characteristics

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Background and Objectives: A previous study of ours indicated that platelet-derived growth factor-A (PDGF-A) mRNA expression in biopsy specimens can identify a subgroup of high-risk gastric carcinoma patients, while clinicopathologic studies have shown that lymph node involvement is an important risk factor for predicting overall survival. To identify gastric carcinoma patients at high risk for recurrence, we assessed a two-step evaluation consisting of mRNA expression of tumor growth–related factors and the histopathologic findings.
Methods: The reverse transcriptase–polymerase chain reaction (RT-PCR) was used to assay the gene expression of PDGF-A and transforming growth factor-β1 (TGF-β1) in 69 gastric carcinoma endoscopic biopsy specimens (prospective cohort). The corresponding gastric carcinoma surgical specimens were classified histologically. Finally, the patients' survival curves were calculated. The relationships among the mRNA expression, histopathologic findings, and survival period were analyzed statistically.
Results: Nodal involvement correlated with PDGF-A and TGF-β1 mRNA expression in early and advanced carcinomas, respectively. Both PDGF-A mRNA and TGF-β1 mRNA expression were independent preoperative prognostic indicators in advanced cases. The ratio of involved nodes (n1) to total perigastric lymph nodes dissected (percentage of involved nodes) was the most independent postoperative prognostic indicator in advanced cases. Early carcinomas were divided preoperatively into two types. Advanced carcinomas were divided preoperatively into three. These were divided again postoperatively according to the percentage of involved nodes into high- and low-malignacy groups.
Conclusions: A two-step evaluation of the malignant potential of gastric carcinoma by a combination of preoperative evaluation for PDGF-A and TGF-β1 expression and postoperative pathologic examination would yield a more accurate prognosis for patients with gastric carcinoma.
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KEY WORDS: biopsy specimen; platelet-derived growth factor-A (PDGF-A) mRNA expression; transforming growth factor-β1 (TGF-β1) mRNA expression; nodal involvement

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INTRODUCTION

The surgical resection of gastric carcinomas has been decided primarily on the basis of the endoscopic macroscopic findings and the pathologic features of the biopsy specimens. In addition, the postoperative therapeutic plan often is selected according to the pathologic stage of the surgically resected specimens. In general, it has been thought that the degree of gastric wall invasion and lymph node metastasis are the most independent prognostic factors in both early and advanced stage gastric carcinoma [1–7]. These observations suggested that the accurate preoperative evaluation of the depth of invasion and nodal status may lead to more effective therapeutic procedures. However, an accurate diagnosis of lymph node involvement is difficult even during surgery [8]. In addition, patients with gastric carcinoma who have similar pathologic stages can have different prognoses. These findings have emphasized the need for a more accurate assessment of the malignant potential of each gastric carcinoma.

Recent molecular biologic studies have indicated that the malignant behavior of a gastric carcinoma may be regulated genetically, and that several types of growth factors at the tumor site may be closely related to its malignant characteristics. In a previous study of ours, the mRNA expression of eight different tumor growth–related factors, comprising cyclin D1, cyclin E, urokinase-type plasminogen activator, 72 kDa type IV collagenase, vascular endothelial growth factor, platelet-derived growth factor-A (PDGF-A), transforming growth factor-β1 (TGF-β1), and interleukin-10, were examined in endoscopic biopsy gastric carcinoma specimens [9]. The relationships among the mRNA expression of these genes, clinical pathologic parameters, and survival period were analyzed statistically [10]. The expression of PDGF-A mRNA was a preoperative prognostic indicator in advanced cases [10]. In this study, we demonstrate that nodal involvement correlates with PDGF-A mRNA and TGF-β1 mRNA expression in early and advanced cases, respectively, and that the grade of involved perigastric lymph nodes in surgically resected specimens is a more potent postoperative prognostic indicator than mRNA expression of PDGF-A or TGF-β1 in advanced cases. We advocate a new therapeutic strategy based on a two-step evaluation of the malignant potential of a gastric carcinoma by a combination of its molecular biologic (preoperative) and clinical pathologic (postoperative) characteristics.

MATERIALS AND METHODS

Patients and Biopsy Samples

The tumor biopsy specimens were obtained during preoperative endoscopy of 69 patients with gastric carcinoma who underwent resection at the Department of Surgery, Saga Medical School, between 1993 and 1996 (prospective cohort study). Each specimen was divided into two blocks. One was minced in RNA lysis buffer on ice for extraction and subsequent analysis by reverse transcriptase–polymerase chain reaction (RT-PCR) while the other block was fixed in 10% formalin for histopathologic examination. Neoplastic cellularity in the biopsies was >60%. All 69 primary gastric carcinoma surgically resected specimens were classified histologically using the General Rules for Gastric Cancer Study of the Japanese Research Society for Gastric Cancer [11]. Early gastric carcinomas are defined as those in which the invasion is limited to the gastric mucosa or submucosa, regardless of the nodal involvement. There were 34 early cases (t1) and 35 advanced cases (t2 or greater) by histologic staging. According to the Japanese classification scheme, t1, t2, t3, and t4 correspond to tumor invasion of the mucosa or submucosa, muscularis propria or subserosa, the serosa without invasion of adjacent structures, and adjacent structures, respectively. The follow-up period ranged from 4 to 37 months (mean, 22.8).

RT-PCR and Gel Electrophoresis

Total RNA from each biopsy specimen was isolated by a single step, guanidinium thiocyanate–phenol chloroform extraction [12]. The biopsy specimens, which were maintained on ice, were minced and homogenized manually in the presence of lysis buffer. The RNA fraction was resuspended in diethyl pyrocarbonate–treated water and quantified by measuring the absorbance at 260 nm. RT-PCR was carried out according to the Perkin-Elmer Cetus protocol for reverse transcription (RT) of RNA and amplification of cDNA. The RT reaction was carried out with 0.5 μg of RNA per sample. cDNA amplification for biologic malignancy-related factors was performed as shown in Table I. Aliquots of the PCR products (7.5 μL) were separated and visualized with ethidium bromide staining after electrophoresis in a 1.5% agarose gel in Tris-acetate-EDTA buffer at 100 V for 20 min. A positive specimen means that the appropriate band was visualized by ethidium bromide staining. Therefore, a negative result does not necessarily mean no mRNA expression. RT-PCR was performed immediately after sample collection by the same investigator without knowledge of the corresponding clinical data.

Statistics

The χ^2 test was used for statistical analyses between the differential mRNA expression of the tumor growth–related factors and the traditional clinical pathologic parameters. The survival curves were calculated using the Kaplan-Meier method and analyzed by the log-rank test. The influence of each variable on survival was assessed by Cox's proportional hazard model. All calculations

TABLE I. Polymerase Chain Reation (PCR) Primers and Conditions

	Primers	PCR conditions	Expected fragment size (bp)
β-actin	5′: GTG GGG CGC CCC AGG CAC CCA 3′: CTC CTT AAT GTC ACG CAC GAT TTC	94°C for 60 sec, 58°C for 60 sec, and 72°C for 120 sec (35 cycles)	541
PDGF-A	5′: CCC CTG CCC ATT CGG AGG AAG AGA 3′: TTG GCC ACC TGG ACG CTG CGG TG	94°C for 60 sec, 60°C for 60 sec, and 72°C for 120 sec (30 cycles)	228
TGF-β1	5′: AAG TGG ATC CAC GAG CCC AA 3′: GCT GCA CTT GCA GGA GCG CAC	94°C for 60 sec, 65°C for 60 sec, and 72°C for 120 sec (27 cycles)	247

TABLE II. PDGF-A and TGF-β1 mRNA Expression Predict Nodal Involvement

	Early-stage cases (n = 34)			Advanced-stage cases (n = 35)		
	PDGF-A(−)	PDGFA(+)	P	PDGF-A(−)	PDGFA(+)	P
Nodal status						
Positive	0	3	0.020	10	15	0.283
Negative	24	7		6	4	
	TGF-β1(−)	TGF-β1(+)	P	TGF-β1(−)	TGF-β1(+)	P
Nodal status						
Positive	1	2	>0.999	6	20	0.070
Negative	12	19		5	4	

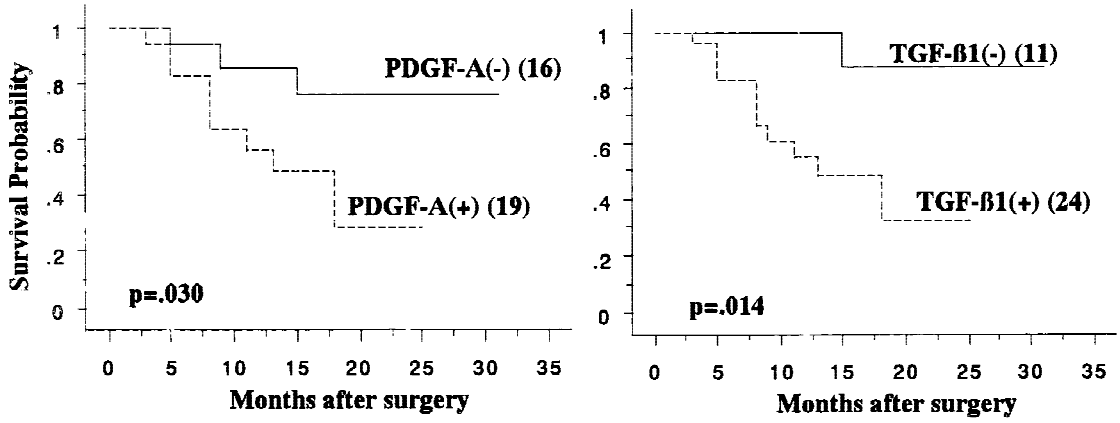

Fig. 1. Survival curves of the 35 patients with advanced-stage gastric carcinoma, according to the expression of PDGF-A mRNA or TGF-β1 mRNA in the carcinoma specimens. P was determined using the log-rank test.

were carried out using StatView (Abacus Concepts, Inc., Berkeley, CA). $P < 0.05$ was considered significant.

RESULTS

Preoperative Evaluation of Malignant Potential

In the 34 patients with early-stage carcinoma, PDGF-A mRNA expression correlated with nodal involvement ($P = 0.020$, Table II). In the 35 patients with advanced-stage carcinoma, TGF-β1 mRNA expression showed a positive correlation with nodal involvement ($P = 0.070$, Table II).

Since none of the 34 early-stage patients died during the follow-up period, correlation analyses between the mRNA expression of the tumor growth–related factors in the biopsy carcinoma specimens and the survival period were performed in the 35 advanced-stage carcinomas. A significant correlation was found between PDGF-A mRNA ($P = 0.030$) and TGF-β1 mRNA expression ($P = 0.014$) and the survival period (Fig. 1).

Postoperative Evaluation of Malignant Potential

The pathologic parameters obtained from resected specimens and the survival period were analyzed for correlation. The presence of lymph node involvement was an important prognostic factor ($P = 0.012$, Fig. 2A). The percentage of involved (n1) perigastric lymph nodes (percentage of positive nodes) was calculated as: % positive nodes = (number of involved nodes/total number of perigastric lymph nodes dissected) × 100. When patients were divided into high-percentage (>30% positive nodes)

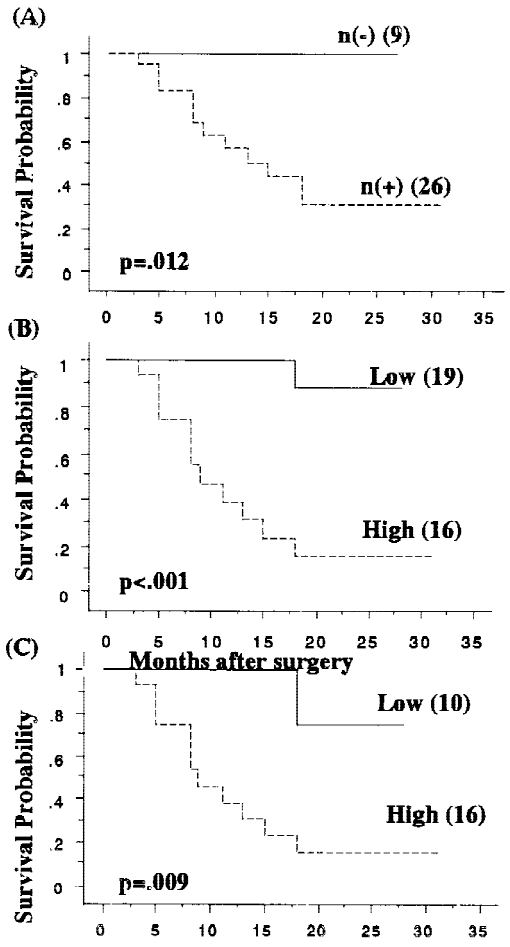

Fig. 2. Survival curves of the 35 patients with advanced gastric carcinoma, according to the presence (n+) or absence (n-) of involved nodes (**A**) or percentage of positive nodes (**B**). The 26 patients with involved nodes were subdivided further, according to the percentage of positive nodes (**C**). The % positive nodes = (number of involved nodes/total number of perigastric lymph nodes dissected) × 100. Low indicates a percentage of positive nodes <30%. High means a percentage of positive nodes >30%.

and low-percentage (≤30% positive nodes) positive-node groups, the prognosis of the group with a high percentage of positive nodes was significantly worse than that of the group with a low percentage of positive nodes ($P < 0.001$, Fig. 2B). The 26 patients with involved nodes were further divided according to the percentage of involved nodes into high- and low-malignancy groups ($P = 0.009$, Fig. 2C). The percentage of positive nodes was a more significant prognostic indicator than the presence or absence of lymph node involvement.

Relationship Between TGF-β1 mRNA Expression and Lymph Node Involvement

PDGF-A mRNA expression and TGF-β1 mRNA expression correlated with the presence of involved nodes in early and advanced carcinoma, respectively (Table II). Next, the most significant tumor growth–related factors affecting percentage of positive nodes were analyzed. In the 35 advanced cases, 3 of the 11 TGF-β1–negative cases had a high percentage of positive nodes and 12 of the 24 TGF-β1-positive cases had a high percentage of positive nodes ($P = 0.187$). The involved nodes in all of the 11 TGF-β1-negative cases were perigastric lymph nodes (n1), but in 14 of the 24 TGF-β1-positive cases, regional lymph nodes outside the perigastric area (n2 or greater) were positive ($P < 0.001$, Fig. 3).

Two-Step Evaluation of Malignant Potential

The results obtained in this study suggested that a two-step evaluation of a gastric carcinoma's malignant potential, that is, both a pre- and postoperative evaluations, may be helpful in designing more effective therapeutic plans in patients with gastric carcinoma.

Early gastric carcinomas can be divided into PDGF-A–negative (low-malignancy cases) and –positive (high-malignancy cases) groups prior to surgery on the basis of the biopsy specimens. All 24 cases classified as low malignancy had neither lymph node involvement nor recurrence. On the other hand, 3 of the 10 cases classified as high malignancy had lymph node involvement, and 1 of these 3 node-positive patients developed multiple bone metastases 12 months after surgery.

Advanced gastric carcinomas were divided into low-malignancy cases if there was neither PDGF-A nor TGF-β1 expression (8 cases), intermediate cases if there was either PDGF-A or TGF-β1 expression (11 cases), and high-malignancy cases if there was expression of both (16 cases) before surgery (Fig. 4).

When the relationship between the mRNA expression of these factors and the percentage of positive nodes was examined, the 8 low-malignancy cases consisted of 5 patients with a low percentage of positive nodes and 3 patients with a high percentage of positive nodes. All of the positive nodes were perigastric lymph nodes (n1). One of the 3 patients with a high percentage of positive nodes died 15 months after surgery.

The 11 intermediate cases consisted of 9 patients with a low percentage of positive nodes and 2 patients with a high percentage of positive nodes; both patients with a high percentagw of positive nodes died 5 and 9 months after surgery. All of the involved nodes in the 3 TGF-β1–negative cases were perigastric lymph nodes. The involved nodes in 4 of the 8 TGF-β1-positive cases extended to regional lymph nodes outside the perigastric area (n2 or greater).

The 16 high-malignancy cases consisted of 6 patients with a low percentage of positive nodes and 10 patients with a high percentage of positive nodes. Eight of the 10 patients with a high percentage of positive nodes died within 18 months after surgery. On the other hand, 2 of the 6 patients with a low percentage of positive nodes

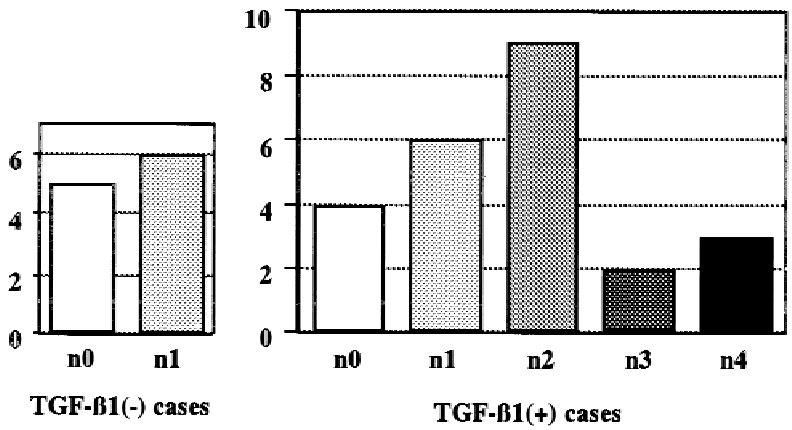

Fig. 3. Relationship between TGF-β1 mRNA expression and the grade of the involved nodes. The involved nodes were classified according to General Rules for Gastric Cancer Study of the Japanese Research Society for Gastric Cancer: n0, n1, n2, n3, and n4 signify the absence of nodal involvement and the presence of group 1, group 2, group 3, and group 4 regional lymph node involvement, respectively. The perigastric lymph nodes belong to group 1 (n1).

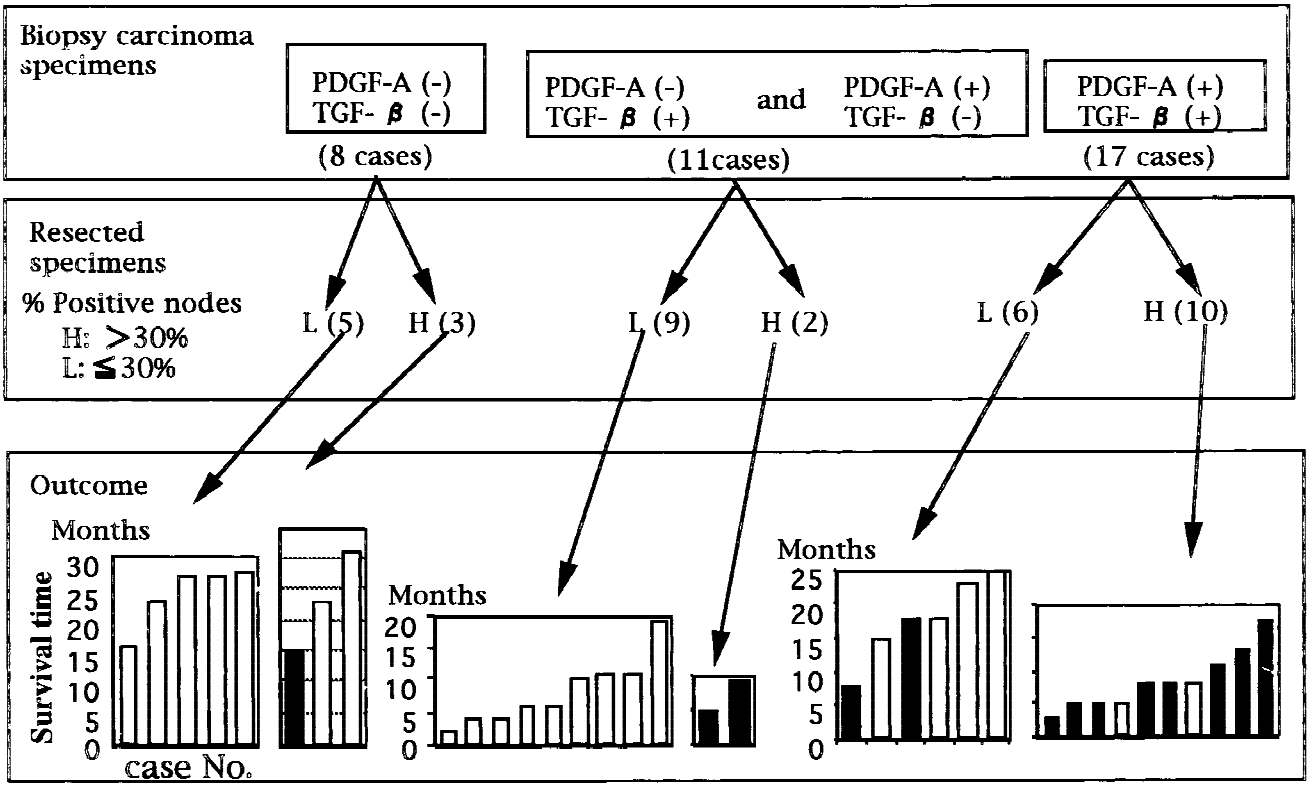

Fig. 4. Classification based on PDGF-A and TGF-β1 expression, percentage of positive nodes, and survival period. L = low percentage of positive nodes; H = high percent age of positive nodes; open square = alive; closed square = dead.

died 8 and 18 months after surgery. Thirteen of the 16 cases had lymph node involvement, and the involved nodes in 10 of the 13 node-positive cases extended to regional lymph nodes outside the perigastric area.

DISCUSSION

Accurate pre-, intra-, and postoperative evaluation of the malignant characteristics of gastric carcinoma is required for the proper application of more effective therapies. We have proposed a new therapeutic strategy for gastric carcinoma based upon two-step evaluation of the malignant potential of a tumor by a combination of molecular biologic techniques and traditional clinicopathologic examination.

It generally has been accepted that there is a significant difference in the prognoses of patients with gastric car-

cinoma who have similar traditional clinicopathologic stages. Of the traditional clinicopathologic parameters, lymph node involvement seems to be among the more important risk factors for predicting overall survival [1–7]. For this reason, an extended gastrectomy including D2 or D3 lymph node dissection has been recomended in Japan for better survival [7,13,14]. Even after a curative resection, however, a subset of patients will have disease recurrence within a few years after surgery [15]. These findings have strongly suggested that gastric carcinomas of a given clinical stage include at least two subgroups with low and high malignant potential.

Our previous studies have demonstrated that PDGF-A mRNA expression in gastric carcinoma specimens is an important prognostic parameter independent of the clinicopathologic parameters [10]. In the present study, we demonstrated that TGF-β1 mRNA expression and PDGF-A mRNA expression are independent prognostic indicators in advanced gastric carcinoma (Fig. 1). The overall results obtained in our studies suggest that the preoperative evaluation of the malignant characteristics of a tumor by molecular biologic techniques would aid in selecting more effective surgical approaches for gastric carcinoma patients.

It has been generally accepted that lymph node involvement is the most important prognostic indicator in patients with early gastric carcinoma [4,6,16]. Our study suggests that PDGF-A mRNA expression in carcinoma biopsy specimens may be an indicator of nodal involvement (Table II). Thus, early carcinomas can be divided into two groups, a PDGF-A–negative group (low-malignancy group) and PDGF-A–positive group (high-malignancy group). It has been reported that lymph node involvement determined by routine hematoxylin-eosin staining was found in 10–15.7% of early gastric carcinomas [6,17–20]. Some investigators have reported that the overall incidence of lymph node involvement is >20% when more careful scrutiny is performed [21,22]. In our study, only 3 (8.8%) of 34 cases had positive nodal involvement by routine staining. In 1 of the 7 PDGF-A–expressing node-negative cases, micrometastases were identified by a detailed reexamination. It is possible that occult lymph node involvement exists in PDGF-A–expressing node-negative cases [23]. Therefore, we now recommend D1+α or D2 lymph node dissection for an absolute curative operation in PDGF-A–expressing cases.

It has been well documented that the depth of gastric wall invasion and lymph node involvement are the most independent prognostic factors in advanced cases [1–7]. We can now estimate with relative accuracy the depth of invasion prior to surgery, and especially during surgery [8]. However, it is still difficult to evaluate accurately the extent of lymph node involvement during surgery [24,25]. To evaluate the extent of lymph node involvement prior to surgery, the relationship between the mRNA expression of tumor growth–related factors and nodal status defined histologically was analyzed statistically. The expression of TGF-β1 mRNA correlated with the presence of lymph node metastasis, suggesting a possible preoperative indicator of nodal involvement (Table II).

In our study, a correlation analysis between lymph node involvement and the survival period also was performed. Interestingly, the ratio of involved nodes to total nodes dissected was a more significant prognostic indicator than the presence or absence of involved nodes (data not shown). Similar observations have been reported previously [1,14,25,26]. Therefore, it is accepted that extensive lymph node dissection is effective in patients with advanced gastric carcinoma in Japan [11,14,27]. Although this approach has not necessarily been adopted outside of Japan [28,29], the need for the dissection of the perigastric lymph nodes (n1 group) has been accepted worldwide. Thus, we carefully examined the status of the perigastric lymph nodes but not all of the lymph nodes resected. We analyzed statistically the relationship between the ratio of involved nodes to total perigastric lymph nodes resected and the survival period. We found that the ratio of involved nodes (percentage of positive nodes) was a more significant prognostic indicator than the presence of involved nodes (Fig. 2). In addition, TGF-β1 mRNA expression correlated well with the grade of node involvement (Fig. 3). Therefore, we recommend a more extensive lymph node dissection for TGF-β1-positive patients compared with TGF-β1-negative patients.

PDGF-A also was an important prognostic factor (Fig. 1), and multivariate analysis demonstrated that TGF-β1 and PDGF-A were independent prognostic factors (data not shown). Thus, advanced carcinomas can be divided into three groups according to the mRNA expression of TGF-β1 and PDGF-A (Fig. 4). The prognosis of patients with both TGF-β1 and PDGF-A expression was extremely poor, and 10 of the 16 patients died of recurrence within 18 months after surgery (Fig. 4). These results stressed the need of some systemic adjuvant treatments for better prognosis. However, any adjuvant chemotherapy has not been shown to be useful. It is possible that extended gastrectomy including D3 lymph node dissection should be avoided for such widespread cases until useful adjuvant treatments were discovered. We found that only 1 of 7 patients with neither TGF-β1 nor PDGF-A expression had developed recurrent disease 15 months after surgery. This patient had a high percentage of positive nodes. None of the 5 patients with a low percentage of positive nodes died during the study period. It is thought that lymph node involvement is an important risk factor for hematogeneous spread [14,30–32]. We postulate that metastases to many lymph nodes (high percentage of positive nodes) reflect a highly in-

vasive carcinoma with a high number of hematogeneous metastases.

In summary, the malignant potential of early gastric carcinomas can be evaluated according to their PDGF-A mRNA expression prior to surgery (first-step evaluation) and nodal status following surgery (second-step evaluation). We postulate that PDGF-A–expressing carcinomas possess malignant characteristics similar to those found in patients with systemic disease. The malignant potential of advanced gastric carcinomas can be evaluated according to their PDGF-A mRNA and TGF-β1 mRNA expression prior to surgery (first-step evaluation) and the percentage of positive perigastric lymph nodes after surgery (second-step evaluation). We speculate that advanced carcinomas with either PDGF-A or TGF-β1 expression possess biologic characteristics similar to systemic disease rather than local disease. In addition, since TGF-β1–expression correlated well with the ability of the tumor to spread to lymphatic tissue, extensive lymph node dissection should be considered in patients with TGF-β1-expressing carcinoma. We believe that the metastatic ability of a gastric carcinoma to spread to many lymph nodes reflects the highly malignant potential of a carcinoma.

Although investigations continue regarding the kinds of adjuvant therapies that should be supplemented [5,26,33–37], two-step evaluations of biologic malignant characteristics may lead to more effective therapeutic strategy for gastric carcinoma.

REFERENCES

1. Kim JP, Yang HK, Oh, ST: Is the new UICC staging system of gastric cancer reasonable? Comparison of 5-year survival rate of gastric cancer by old and new UICC stage classification. Surg Oncol 1992;1:209–213.
2. Moriguchi S, Hayashi Y, Nose Y, et al.: A comparison of the logistic regression and the Cox proportional hazard models in retrospective studies on the prognosis of patients with gastric cancer. J Surg Oncol 1993;52:9–13.
3. Marubini E, Bonfanti G, Bozzetti F, et al.: A prognostic score for patients resected for gastric cancer. Eur J Cancer 1993;29A:845–850.
4. Shiozawa N, Kodama M, Chida T, et al.: Recurrent death among early gastric cancer patients: 20-years' experience. Hepatogastroenterology 1994;41:244–247.
5. Kim JP, Kim YW, Yang HK, Noh DY: Significant prognostic factors by multivariate analysis of 3926 gastric cancer patients. World J Surg 1994;18:872–877.
6. Kim JP, Hur YS, Yang HK: Lymph node metastasis as a significant prognostic factor in early gastric cancer: analysis of 1,136 early gastric cancers. Ann Surg Oncol 1995;2:308–313.
7. Noguchi M, Miyazaki I: Prognostic significance and surgical management of lymph node metastasis in gastric cancer. A review. Br J Surg 1996;83:156–161.
8. Korenaga D, Okuyama T, Orita H,et al.: Role of intraoperative assessment of lymph node metastasis and serosal invasion in patients with gastric cancer. J Surg Oncol 1994;55:250–254.
9. Nakamura M, Katano M, Fujimoto K, Morisaki T: A new prognostic strategy for gastric carcinoma: mRNA expression of tumour growth-related factors in endoscopic biopsy specimens. Ann Surg 1997;226:35–42.
10. Katano M, Nakamura M, Fujimoto K, et al.: Prognostic value of platelete-derived growth factor A (PDGF-A) in gastric carcinoma. Ann Surg 1998;227:365–371.
11. Japanese Research Society for Gastric Cancer: The general rules for gastric cancer study. Jpn J Surg 1985;11:127–139.
12. Chromcznski P, Sacchi N: Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Ann Biochem 1987;162:156–159.
13. Noguchi Y, Imada T, Matsumoto T, et al.: Radical surgery for gastric cancer: a review of the Japanese experience. Cancer 1989; 64:2053–2062.
14. Isozaki H, Okajima K, Kawashima Y, et al.: Prognostic value of the number of metastatic lymph nodes in gastric cancer with radical surgery. J Surg Oncol 1993;53:247–251.
15. Miwa K: Evaluation of TNM classification of stomach cancer and proposal for its rational stage-grouping. Jpn J Clin Oncol 1984; 14:385–410.
16. Maehara Y, Okuyama T, Oshiro T, et al.: Early carcinoma of the stomach. Surg Gynecol Obstet 1993;177:593–597.
17. Maehara Y, Orita H, Okuyama T, et al.: Predictors of lymph node metastasis in early gastric cancer. Br J Surg 1992;79:245–247.
18. Nakamura K, Ueyama T, Yao T, et al.: Pathology and prognosis of gastric carcinoma. Findings in 10,000 patients who underwent primary gastrectomy. Cancer 1992;70:1030–1037.
19. Norii A, Sakai M, Inoue K, et al.: A clinicopathological analysis of early gastric cancer: retrospective study with special reference to lymph node metastasis. Cancer Detect Prev 1994;18:437–441.
20. Yamao T, Shirao K, Ono H, et al.: Risk factors for lymph node metastasis from intramucosal gastric carcinoma. Cancer 1996;77: 602–606.
21. Natsugoe S, Aikou T, Shimada M, et al.: Occult lymph node metastasis in gastric cancer with submucosal invasion. Surg Today 1994;24:870–875.
22. Siewert JR, Kestlmeier R, Busch R, et al.: Benefits of D2 lymph node dissection for patients with gastric cancer and pN0 and pN1 lymph node metastases. Br J Surg 1996;83:1144–1147.
23. Maehara Y, Oshiro T, Endo K,et al.: Clinical significance of occult micrometastasis in lymph nodes from patients with early gastric cancer who died of recurrence. Surgery 1996;119:397–402.
24. Adachi Y, Mori M, Maehara Y, Sugimachi K: Macroscopically node-negative but histologically node-positive gastric carcinoma. Br J Surg 1995;82:1254–1256.
25. Matsushita M, Hajiro K, Suzaki T, et al.: Histopathological assessment of lymph node metastasis in patients with gastric cancer. Hepatogastroenterology 1995;42:861–866.
26. Kim JP, Kwon OHJ, Oh ST, Yang HK: Results of surgery on 6589 gastric cancer patients and immunochemosurgery as the best treatment of advanced gastric cancer. Ann Surg 1992;216:269–279.
27. Mok YJ, Koo BW, Whang CW, et al.: Cancer of the stomach: a review of two hospitals in Korea and Japan. World J Surg 1993; 17:777–782.
28. Siewert JR, Bottcher J, Roder JD, et al.: Prognostic relevance of systemic lymph node dissection in gastric carcinoma. Br J Surg 1993;80:1015–1018.
29. Bonenkamp JJ, Songun I, Hermans J, et al.: Randomised comparison of morbidity after D1 and D2 dissection for gastric cancer in 996 Dutch patients. Lancet 1995;345:745–748.
30. Moriguchi S, Maehara Y, Korenaga D, et al.: Risk factors which predict pattern of recurrence after curative surgery for patients with advanced gastric cancer. Surg Oncol 1992;1:341–346.
31. Maehara Y, Tomisaki S, Oda S, et al.: Lymphatic advancement to peritoneal dissemination and liver metastasis in gastric cancer patients. Anticancer Res 1994;14:2755–2757.
32. Maehara Y, Oshiro T, Baba H, et al.: Lymphatic invasion and potential for tumor growth and metastasis in patients with gastric cancer. Surgery 1995;117:380–385.

33. Hattori T, Mori A, Hirata K, Ito I: Five-year survival rate of gastric cancer patients treated by gastrectomy, a high-dose of mitomycin-C and/or allogeneic bone marrow transplantation. Gann 1978;63:517–522.
34. The Gastrointestinal Tumor Study Group: A comparative clinical assessment of combination chemotherapy in the management of advanced gastric carcinoma. Cancer 1982;49:1362–1366.
35. Okumura T, Korenaga D, Baba H, et al.: Postoperative adjuvant chemotherapy inhibits early recurrence of early gastric carcinoma. Cancer Chemother Pharmacol 1989;23:319–322.
36. Suzuki T, Ochiai T, Nagata M, et al.: High-dose chemotherapy with autologous bone marrow transplantation in the treatment of advanced gastric cancer. Cancer 1993;72:2537–2542.
37. Averbach AM, Jacquet P: Strategies to decrease the incidence of intra-abdominal recurrence in resectable gastric cancer. Br J Surg 1996;83:726–733.